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Phylloporus brunneiceps from Pakistan

A. Naseer[1]*, A.N. Khalid[2] & A.R. Niazi[2]

[1] *Centre for Undergraduate Studies &* [2] *Department of Botany, University of the Punjab, Quaid-e-Azam Campus-54590, Lahore, Pakistan*

* Correspondence to: *arooj.hons@pu.edu.pk*

Abstract—The fungal species *Phylloporus brunneiceps* (*Boletaceae, Boletales*) from a pure *Quercus* forest is described and illustrated as a new record for Pakistan. Phylogenetic analyses, description, photographs, and line drawings of the Pakistani collection are provided.

Key words—gilled boletes, ITS, oak, 28S, rDNA

Introduction

Phylloporus Quél. is a gilled bolete represented by the type species *Agaricus pelletieri* Lév. [≡ *P. pelletieri* (Lév.) Quél.] from temperate Europe. The genus encompasses a diverse group characterized by predominantly lamellate instead of poroid hymenophores (Singer 1986, Binder & Bresinsky 2002, Neves & Halling 2010, Neves et al. 2012, Zeng et al. 2013). It forms mycorrhizal associations with members of *Casuarinaceae*, *Dipterocarpaceae*, *Fabaceae* (*Caesalpinioideae*, *Mimosoideae*), *Fagaceae*, *Myrtaceae,* and *Pinaceae* (Heinemann & Rammeloo 1987, Halling & Mueller 2002). The moderately small genus known represented worldwide (Neves & Halling 2010; Neves et al. 2012; Zeng et al. 2013) by 90 species plus several subspecies and varieties (https://www.indexfungorum.com; accessed July 2017).

Phylloporus is regarded as more abundant in Malaysia and Australia, where most species have been described (Corner 1970, 1974; Watling 2008). However, *Phylloporus* and other boletes have not been well studied in many areas, and new records are frequently reported (Li et al. 2011; Zeng et al. 2011, 2013; Hosen & Li 2017).

Only *P. rhodoxanthus* (Schw.) Bres. has been reported from Pakistan, based on morphological evidence (Sultana et al. 2011). We describe a second *Phylloporus* species from Pakistan, *P. brunneiceps,* based on morphological and ITS1-5.8S-ITS2 and 28S rDNA sequence analyses. *Phylloporus brunneiceps* is associated with *Quercus* in moist temperate forests of Pakistan.

Material & methods

Sampling site

The specimen was collected during a field investigation of ectomycorrhizal communities associated with oak forests near Alpurai forest. The collection area, Toa (34°54′N 72°39′E), lies on the boundary of Shangla and Swat districts (KPK province, Pakistan). The region, which ranges in elevation from 850 to 2350 m, is characterized by a very prolonged cold winter, short summer, and annual rainfall of approximately 142 cm.

Morphological analyses

The specimen was photographed in field using a Nikon D70S digital camera. After the odor and color change upon bruising were recorded, the specimen was wrapped in aluminum foil and kept separately in a collection box. Colors were designated with reference to mColorMeter. After being dried by a fan heater, the collection was sealed in plastic and deposited in Lahore Herbarium, Department of Botany, University of the Punjab, Lahore, Pakistan (LAH).

Tissues from lamellae, pileipellis and stipitipellis were mounted in phloxine (1%) for better contrast, Melzer's reagent for amyloid basidiospore ornamentation, and KOH (5%) for colored hyphae and examined using a Meiji Techno MX4300H compound microscope. Measurements were determined for basidiospores, basidia, and other anatomical cells under the light microscope equipped with a camera lucida.

DNA extraction, amplification and sequencing

DNA was extracted from basidiome gills following modified CTAB method (Bruns 1995). PCR amplification was performed with primer pairs ITS1F/ITS4 (White et al. 1990) for the ITS region, and LR0R/LR7 (Vilgalys & Hester 1990) for the 28S region of nuclear ribosomal DNA. PCR conditions for ITS and 28S were as follows: an initial denaturation step at 94°C for 4 min, followed by 34 cycles of 94°C for 40 sec, 55°C (ITS) or 52°C (28S) for 40 sec, and 72°C for 1 min, and a final elongation step at 72°C for 8 min. The PCR products were sent to Macrogen Inc. (Korea) for sequencing. Sequences were submitted to GenBank.

Molecular phylogenetic analyses

The consensus sequences for ITS and 28S were generated from the sequences obtained by both the primers using BioEdit software. GenBank sequences of closely related taxa were used to prepare a ITS and 28S data matrix based on a BLAST

Fig. 1. *Phylloporus brunneiceps* (LAH35203). Basidiomata. Scale bars = 10 mm.

search. The downloaded sequences and newly generated sequences were aligned using MUSCLE v3.6 (Edgar 2004) and manually adjusted where necessary.

The ITS sequences were trimmed with the conserved motifs. The alignment portion between 5′...GAT and CAAA...3′ was included in phylogenetic analysis. Maximum likelihood (ML) analysis was performed using a general time-reversible model (Nei & Kumar 2000) and nearest-neighbor interchange as the ML heuristic search method in MEGA6 software to test the phylogeny at 1000 bootstraps.

Results

Taxonomy

Phylloporus brunneiceps N.K. Zeng, Zhu L. Yang & L.P. Tang, Fung. Divers. 58: 82 (2013) Figs 1,2

Basidiomata medium-sized. Pileus 7.5 cm diam., centrally depressed, surface dry, tomentose, dark brown (9YR 1.5/1.7) in centre, brown (2Y 3.8/3.1) near the margin; margins inrolled. Lamellae ≤0.5 cm high, decurrent, subdistant, bright yellow (5.3Y 6.1/8.9) to yellow (3.7Y 3/5.6), becoming blue when injured before reverting to the original yellow. Lamellulae present, variable in size, in one tier. Stipe 6.5 × 1.2 cm, central, cylindric, solid, dry, tomentose, yellowish white or greenish yellow (1.1GY 7.6/2.9) at base, becoming yellow (7.5Y 7.2/4.8) in middle and bright orange or yellow red (6YR 3.1/7.4) at top. Annulus absent.

Basidiospores [n/b/p = 30/1/1] (10.2–)10.3–13.7(–14) × (3.7–)4.0–4.6 (–4.7) µm, mean = 11.69 × 4.45 µm, Q = (2.13–)2.23–2.97(–3), avQ = 2.85, ellipsoid, smooth, thick-walled, olive brown in KOH, dextrinoid. Basidia 33–43 × 8–9 µm, clavate, thin-walled, 4-spores, sterigmata 4–5 µm in length. Cheilocystidia 30–50 × 11–14 µm, subclavate, thin-walled, light yellow in KOH. Pleurocystidia 69–100 × 10–16 µm, fusiform, thick-walled, light yellow in KOH, abundant. Pileipellis a trichoderm, hyphae 4–10 µm diam., yellowish brown in KOH, rarely branched, end cells obtuse. Stipitipellis a trichoderm-like structure composed of thin-walled hyphae, light yellow in KOH. Clamp connections absent.

Material examined: **PAKISTAN, Khyber Pakhtunkhwa Province, Shangla/ Swat boundary,** Toa, 2100 m asl, solitary on ground under *Quercus incana* Roxb. [= *Q. oblongata* D. Don], 15 July 2015, Arooj Naseer & Abdul Nasir Khalid AST44(LAH35203, GenBank KY679591, KY679592 & KY679593).

Molecular phylogeny

Sequencing of the *P. brunneiceps* PCR products using ITS1F/ITS4 yielded 690 base pairs, trimmed to a 640-base pair consensus sequence. The BLAST

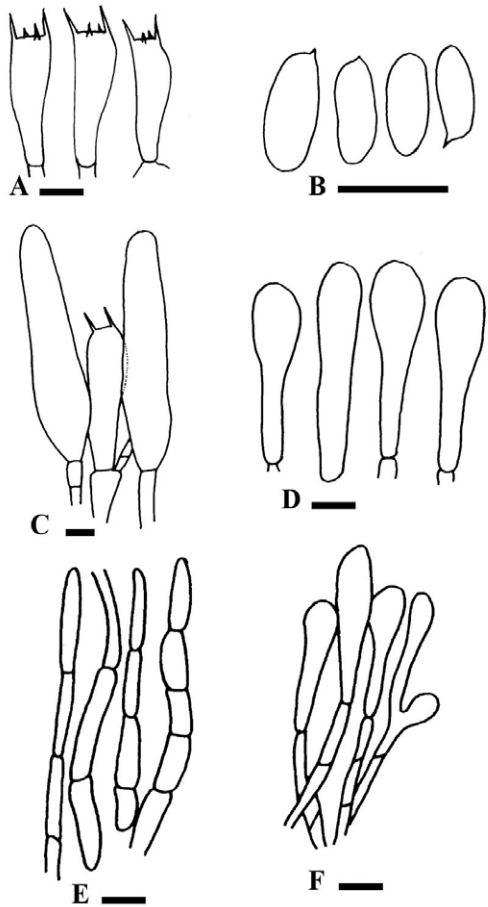

Fig. 2. *Phylloporus brunneiceps* (LAH35203).
A. basidia; B. basidiospores; C. pleurocystidia; D. cheilocystidia; E. pileipelli; F. stipitipellis.
Scale bars = 10 μm.

search revealed LAH35203 as 98% identical with JQ967242, JQ967244, (100% query cover, 0.0 E value) and a 97% identity with KJ609173 and JQ967243 (99% query cover, 0.0 E value).

Sequencing of the *P. brunneiceps* PCR products using LROR/LR5 yielded 840–860 base pairs, trimmed to a 723 base pair consensus sequence. Initial BLAST analysis revealed LAH35203 as 99% identical to JQ967201, NG042664, and JQ967200 (100% query, 0.0 E value).

The sequences were aligned with ITS and 28S sequences of the other related taxa. The final data set included 31 ITS sequences and 30 28S sequences. The analysis clustered the sequences generated during this study with sequences of *P. brunneiceps* from China (Figs 3,4).

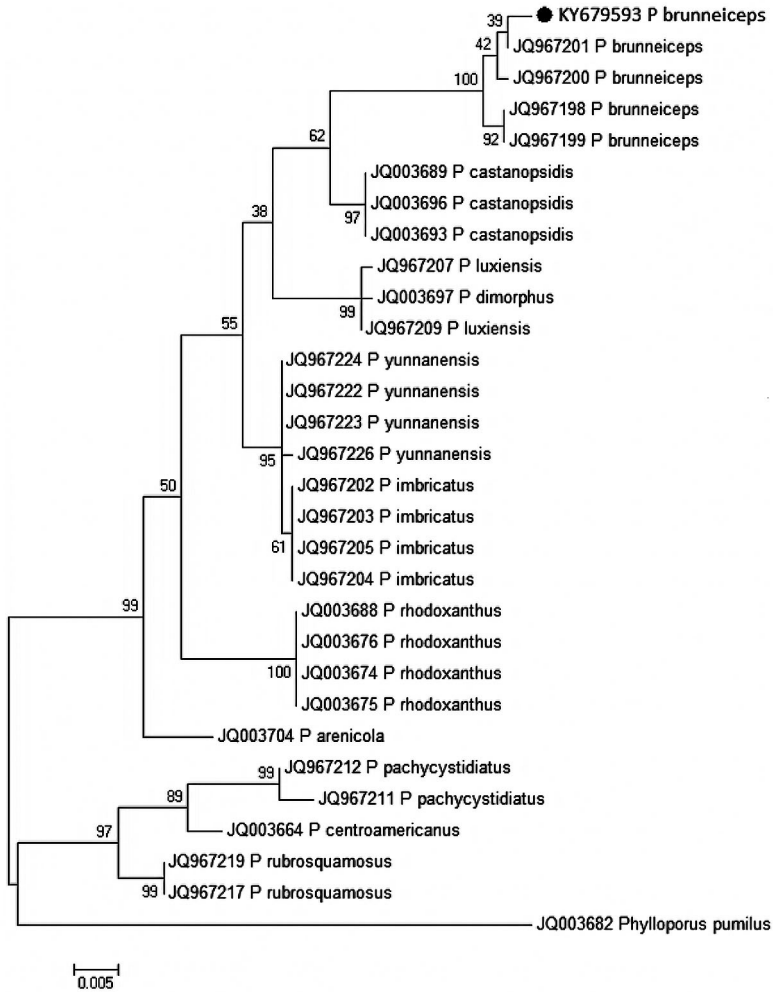

Fig. 3. Molecular phylogenetic analysis of *Phylloporus* based on LSU sequences inferred by the Maximum Likelihood method. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree for the heuristic search was obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated by the Maximum Composite Likelihood (MCL) approach. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. A new sequence generated from Pakistan are marked with ●.There were a total of 782 positions in the final dataset.

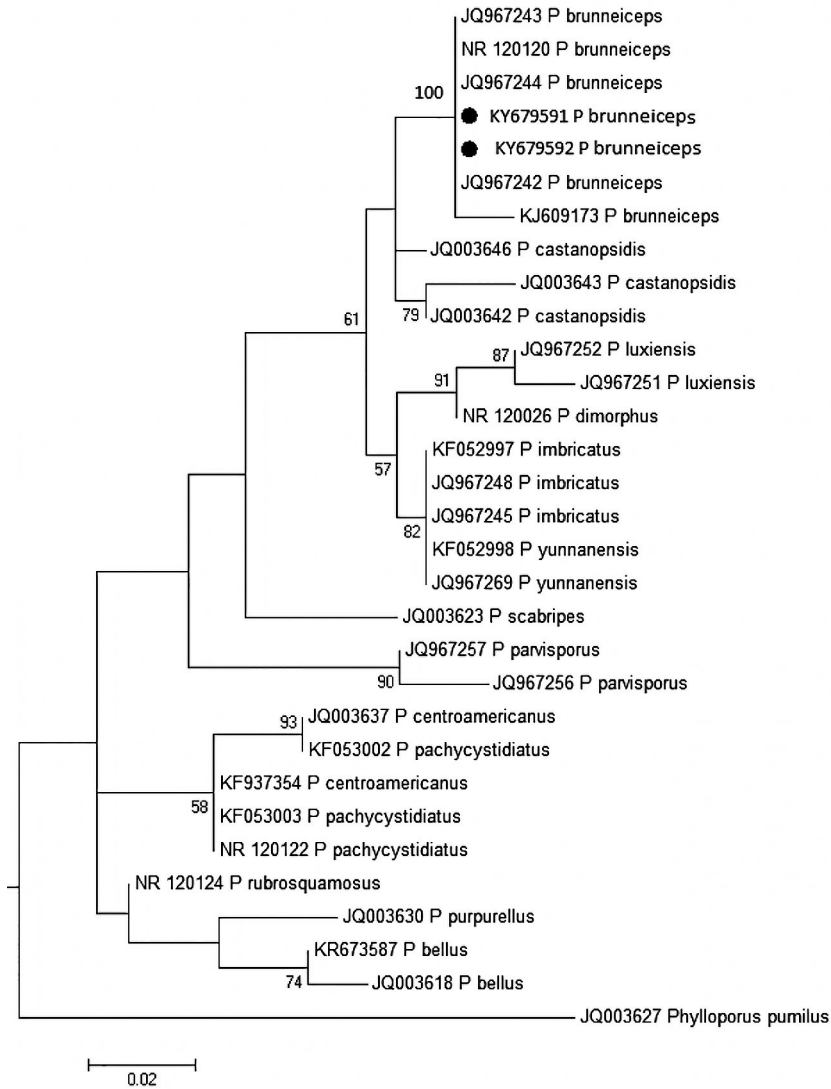

Fig. 4. Molecular phylogenetic analysis of *Phylloporus* based on ITS sequences inferred by the Maximum Likelihood method. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree for the heuristic search was obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated by the Maximum Composite Likelihood (MCL) approach. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. New sequences generated from Pakistan are marked with ● There were a total of 184 positions in the final dataset.

Discussion

Phylloporus brunneiceps is a gilled bolete that is easily recognized in field due to its lamellate hymenophore. It is distinguished by bright yellow decurrent lamellae that change colour when bruised or injured, a brown to dark brown tomentose pileus, and central stipe. Our phylogenetic analysis clustered the ITS and LSU sequences from the Pakistan specimen with the *P. brunneiceps* sequences reported by Zeng et al. (2013). In phylogenetic analysis, *P. brunneiceps* forms a clade with *P. castanopsidis* M.A. Neves & Halling, which is distinguished morphologically by its pale, convex to plano-depressed, dry pileus (Neves et al. 2012).

The Chinese and Pakistani collections of *Phylloporus brunneiceps* differ somewhat morphologically from the Chinese collection, described with a smaller (4–5 cm diam.) pileus and shorter, more slender stipe (3–4 × 0.4–0.7 cm; Zeng et al. 2013).

Previously, *P. brunneiceps* was misidentified in China as *P. rhodoxanthus* (Zang et al. 1996), which differs by its non-staining lamellae and cinnamon brown pileus (Neves & Halling 2010).

Phylloporus brunneiceps has been reported from mixed *Lithocarpus-Pinus* forests in China, while in our study the species has been observed under canopy of *Quercus oblongata* in pure oak forests.

This is the first report of *P. brunneiceps* from Pakistan and so represents an addition to the mycobiota of Pakistan. The species has great potential for mycorrhization in afforestation and reforestation of *Quercus* species.

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